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President : Umetarō SUZUKI.

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Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japaneses texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Umetarō SUZUKI.
Associate Editors : Kakuji GOTŌ and Yoshihiko MATSUYAMA.

A NEW METHOD OF COLORIMETRIC ESTIMATION OF HISTIDINE. PART II.

By Kôzô Suzuki and Yoshio Kaishio.

(From the Chemical Laboratory of Imperial Zootechnical Experiment Station, Chiba.)

(Received Feb. 2nd., 1927.)

In our previous communication (this Jour. Vol. 2, 77, 1926), we have made a report on a method of colorimetric determination of a small amount of histidine by application of Totani's reaction. Since then we have tried to improve the method and succeeded in discovering a new sensitive reaction giving the same permanent golden yellow colouration, not by going all through the complicated Totani's reaction, but by simply treating a histidine solution with sulfanilic acid, making acidic with sulfuric acid, and then making it alkaline by caustic soda or sodium carbonate.

This reaction has been utilized for the determination of histidine with very satisfactory result.

Totani's reaction for histidine consists in carrying out diazo-reaction first and then reducing the product with strong hydrochloric acid and zinc dust, but diazobenzene-sulfonic acid becomes hydrazine or decomposes into sulfanilic acid and ammonia by reduction, while amino acids are generally not reducible in their free states, it being only reduced to aldehyde, when their esters are strongly cooled and treated with natrium amalgam.

Assuming, therefore, that Totani's reaction is, after all, the reaction between histidine and sulfanilic acid, we have proceeded as follows:

So an aqueous solution of histidine is added (a) some excess of 2 % sulfanilic acid solution in 10 % sulfuric acid, and (b) concentrated sulfuric acid enough to make its concentration in the solution 11–15 %. When the solution is well shaken and then made alkaline by the addition of caustic soda or sodium carbonate, the solution gives a golden yellow colour just as in the case of Totani's reaction.

This reaction is applicable to the estimation of a small amount of histidine for following reasons:

(1) This colouring reaction is sensitive and characteristic to histidine. However as phenol gives rise to a similar colouration, the method cannot be used for either detection or estimation of histidine in presence of phenol.

(2) The golen yellow colour by this reaction is permanent for a considerable time.

(3) When the concentration of sulfuric acid is made 11–15 %, the excess of other reagents used, i. e. sulfanilic acid, caustic soda or sodium carbonate, do not affect the intensity of colouration in the slightest degree.

(4) The intensity of the golden yellow colour is directly proportional to the amount of histidine.

(5) The colour can be compared with that of 1/100 normal potassium bichromate solution, as standard.

The relation between the amount of histidine present and the thickness of the layer of potassium bichromate standard solution is exactly the same, as in our previous report, i. e.,

1 mm. layer of $K_2Cr_2O_7$ = 0.4028 mgs. of histidine.
〃 = 0 019032 mgs. of histidine nitrogen.

The computation of unkown amount of histidine can, therefore, be made by mean of the followiding equation:

$$X = \frac{0.40248 \times d}{D}$$

$$X' = \frac{0.010932 \times d}{D}$$

where X = mg. of histidine in 1 c.c.
X' = mg. of histidine nitrogen in 1 c.c.
D = number of m.m. of the histidine solution.
d = number of m.m. of 1/100 normal $K_2Cr_2O_7$ solution

As stated above, phenol gives a similar colouration to the solution when subjected to this reaction, so this method is inapplicable to the estimation of histidine when phenol is present, but there could be no phenol among the decomposition products of proteins, nor any substance which may similarly colour the solution through the same treatment, we can very safely apply this reaction to the estimation of histidine in proteins. Besides our new method has further advantage over Totani's reaction, as by the latter, it is necessary to separate previously hexon bases by mean of phosphotungstic acid, because through Totani's reaction, tyrosine gives a pink colour to the solution, whereas, by our new method, even tyrosine gives no colour, so that we can directly proceed to determine the amount of histidine in hydrolyzed solution of proteins, after its decolourization by mean of charcoal.

Furthermore, when Van Slyke's method is used, at least 3–6 grms of proteins is required for the quantitative determination of histidine in it, but if our method is adopted a small quantity less than 1 g. of protein is ample, and by very simple treatment, as described above, a very satisfactory result could be quickly obtained.

ON THE PHYSIOLOGY OF RHIZOPUS SPECIES.

PART I.

THE RELATION BETWEEN THE CONDITIONS OF THE GROWTH OF THE FUNGUS AND THE PRODUCTION OF ACIDS AND ETHYL ALCOHOL.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received Feb. 15th., 1927.)

The surface growth of the fungus in the culture medium is that one of the commonest and very naturally it is the most favourable one. On the other hand, the growth in the liquid viz. the immersed growth is always inferior to the surface growth. In the former case the production of the acids predominates and in the latter case that of alcohol is more pronounced upon the whole, and in the average by Rhizopus species we observed the fallowings :–

	Surface growth.	Inner growth.
Acidity.	2.15 c.c.	1.62 c.c.
Alcohol. (w. %)	0.34	1.18

The percentage of the increasement of alcohol in the case of the immersed growth over that of the surface growth during eight days was 31 %, which attained to about 128 % after a period of 17 days culture. Even in the case of the immersed growth, if we substitute the fermenting bung instead of cotton plug in the mouth of the flask there occured about one-seventh decrease in acidity and 2% less in alcohol production than the latter case.

Experimental.

Rhizopus G. 34 was used through the experiments.

The first culture medium consited of :– 5g. glucose, 0.15g. $(NH_4)_2SO_4$, 0·008g. KH_2PO_4 and K_2HPO_4, 0.005g. $MgSO_4$ and $CaCl_2$, trace NaCl and Fe_2Cl_6, 50c.c. distilled water.

In 7 days after seeding the growth attained to cover the whole surface of the medium and in that stage about the half of the culture flasks the surface growth was immersed into the liquid with the sterile rod of platinum. After the said treatment, twice in a day, the flasks were shakened gently to set the growth in the immersed state and after a period of 24 days from the begining of the immersed culture, the contents of flasks were analysed.

The temperature of the environment was held at from 25–30°C during the culture. The results of the analyses were :–

		Weight of the growth (g.)	Acidity* c.c. of $^{n}/_{10}$ NaOH to neutralize each 5c.c. of the medium.	Alcohol. w. %
Surface culture.	No. 1.	0.303	2.2	0.32
〃	No. 2.	0.251	2.9	0.72
〃	No. 3.	0.303	2.3	0.22
〃	No. 4.	0.282	—	0.34
〃	No. 5.	0.256	1.9	0.42
Average.		0.286	2.15	0.34
Immersed culture.	No. 6.	0.168	1.5	1.60
〃	No. 7.	0.284	1.7	0.90
〃	No. 8.	0.248	1 6	1.06
〃	No. 9.	0.250	1.7	1.16
Average.		0.237	1.62	1.18

* The acidity of the original medium was. 0.4 c.c.

In the second case $(NH_4)_2SO_4$ in the medium was changed to glycocoll thus ;– 10g. glucose, 0.5g. glycocoll, 0.015g. K_2HPO_4 and KH_2PO_4, 100g. distilled water, and the other ingredients such as $MgSO_4$, $CaCl_2$, NaCl and Fe_2Cl_6 were given in a similar dosed as much as in the first case.

The results of the analyses of the culture at the same temperature as in the first experiment were shown below :–

Cultures.	Days of culture.		Weight of the growth. (g.)	Acidity c.c. of $^{n}/_{10}$ NaOH to neutralize each 5c.c. med.	Alcohol.		Sugar consumed. (g.)
	Before immersion.	After immersion.			w. %	%. of increasement.	
A. a.	21	—	0.701	95.	0.61	100	8.1
b.	21	—	0.751				
B. a.	13	8	0.592	9.0	0.80	131	8.3
b.	13	8	0.612				
c.	13	—	0.510	9.1	0.56	—	—
C. a.	4	17	0.483	5.4	1.38	228	9.0
b.	4	17	0.505				
D.	35	—	0.078	1.3	1.60	98	4.1

[A. Control culture. B. C. Immersed culture. D. The cotton plug was replaced by fermenting bung.]

ON THE PHISIOLOGY OF RHIZOPUS SPECIES.
PART II.

THE RELATIVE QUANTITIES OF THE PRODUCTION OF ETHYL ALCOHOL AND CARBONDIOXIDE.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received Feb. 15th., 1927.)

In the normal viz. surface growth of the culture of Rhizopus species, the ratio of the production of carbondioxide to alcohol is decidedly greater than that of the case of the fermentation by yeast. The cause of the predominancy of the formation of carbondioxide over alcohol must be ascribed to many lines of descriptions. The formation of the rather large quantities of organic acids must be one of these causes and side by side the redecomposition of alcohol produced, into CO_2 and water, must recconed upon as another one. In addition to these causes the redecomposition of fumaric acid into lactic acid and CO_2 could not be neglected as one of them.

In our researches, we selected the special condition, good enough to the atmost production of alcohol. The culture indicated in the part I viz. the immersed culture is one of the most good conditions available for it.

The results are tabulated below :–

Cultures.	Carbon dioxide (g.)		Alcohol. (g.)	CO_2 : C_2H_5OH.	Acidity. c.c. of $^n/_{10}$ NaOH. to neutralize 5c.c. of the medium.	Acid (g.) as fumaric acid.	Volatile acid. Acidity c.c. of $^n/_{10}$ NaOH. for 5c.c. medium.	Sugar consumed. (g.)
	Determ. by w.*	Determ. by KOH-bulb.						
No. 1.	1.435	1.402	1.56	91.98 : 100	8.4	0.053	0.2	3.25
No. 2.	1.294	—	1.34	96.54 : 100	9.6	0.056	—	3.02
No. 3.	1.411	—	1.52	92.83 : 100	7.8	0.049	—	3.15
Average.				93.78 : 100				

* Determined by the difference of the weight of flask before and after the experiment.

For the comparison of these results to that of already known, the table below is given :–

	$CO_2 : C_2H_5OH$.
Mucor racemosus (Kostgtsew(1) and others)	100 : 98
〃 〃	100 : 99
Aspergillus niger.	100 : 91
〃	100 : 92
〃	100 : 94
Yeast. (Paster).	95.3 : 100 [46.4 % : 48.67%]
〃 (Todlbauer).	95.6 : 100 [46.54% : 48.67%]
〃 (Kosutany).	98.9 : 100 [47.50% : 48.08%]

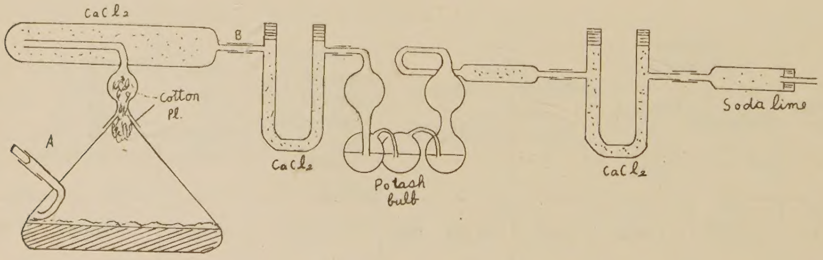
As seen from the tables, the authors are induced to point out that the fungus of Rhizopus species behaves very similar way of the alcoholic fermentation, which takes place by yeast.

Experimental.

Rhizopus species used by this experiment was the same one as in the part I viz. Rhiz. G. 31. Culture medium: glucose 6g., Pepton 0.3g., solution of mineral matters 60c.c.

The solution of mineral matters consisting of; distilled water 1000c.c., K_2HPO_2 and KH_2PO_4 each 0.15g., $CaCl_2$ and $MgSO_4$ each 0.1g., Fe_2Cl_6 and NaCl each trace.

For the culture, a special flask (see the sketch), provided with all appliances suitable for an aseptic working, was used for the benefit of the accurate determination of carbon dioxide evolved during the course of fermentation.



The seeding of the fungus was done from the side tube of the flask (marked A) and kept at the temperature of 25–30°C during two weeks and for the sake of the complete growth of the fungus, twice every day about 20 minutes a fresh air freed from moisture, CO_2, and the germs of microbes is introduced shaking the flask gently in each times. After the complete growth viz. the film covering the whole surface of the medium, the fluid under the film is decanted and washing several times with the sterile distilled water, until there remains any indication of the presence of acids and sugars, culture medium anew is introduced from the side tube as done in the begining.

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(1) Kostytschew u. Andere. Woch. f. Brau. 1926. No. 23, S. 259.

Carbon dioxide evolved was determined from the difference of the weight of the flask, detatched from B, (see the sketch), at the begining of the experiment and the end of the growth of the fungus.

In another case it was absorbed by potash bulb. In either case, to ensure the accuracy of the determination of it, the flask was wormed at about 70°C and a current of air freed from the moisture, CO_2 and the germs of microbes was introduced in to drive off the last traces of the carbonic acid charged in the medium, and in one case it was to be absorbed in the potash bulb.

ON THE PHISIOLOGY OF RHIZOPUS SPECIES.
PART III.

THE EFFECT OF PHOSPHORIC ACID AND POTASSIUM FOR THE PRODUCTION OF ACIDS AND ETHYL ALCOHOL.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received Feb. 15th., 1927.)

It is well known, that in the alcoholic fermentation of yeast the presence of an adequate quantities of both potassium and phosphoric acid in the culture medium activates or favours the growth of the organism to produce alcohol in an atmost quantity. Since this fungus produces acids rather in a large quantity, than pronounced by yeast, the authors proceeded to research on the effect of both potassium and phosphoric acid for the formation of not only for alcohol but also for organic acids, such as fumaric, succinic and lactic acids from glucose by the fungus.

The result attained was that the formation of alcohol by the fungus, Rhizopus G. 34, is rather favourable, when the doses of potassium and phosphoric acid are smaller than that of normal or common medium viz. medium which is the most favourable for the growth. As to the effect of of phosphoric acid on the formation of the acids, just fumaric acid seems to be found in the largest quantity in normal medium; although other acids such as, succinic, malic and lactic acids have not found with the differences worth-mentioning whatsoever in all the cultures with different doses of phosphoric acid. On the other hand, the favourable effect of potassium to increase the production of acids was always ascertained, when we add the less doses of it to the media.

The table below represents the analytical data :-

Culture media.	Quantities of K. and P. in media.	Period of culture. (days).	Total acidity. c.c. of n/10 NaOH to neutral. 5c.c. of medium.	Fumaric acid in 100c.c. medium.				Succinic acid (g.) in 100c.c.	Lactic acid (g.) in 100c.c.	Malic acid (g.) in 100c.c.	Alcohol (g.) in 100c.c.	Sugar remained. (g.)
				As Ca-salt. (g.)	In ether-ext. (g.)	Totol fumaric acid (g.)	Yield against to sugar consum. %					
Normal sol.	K_2HPO_4 0.03g. KH_2PO_4 0.03g.	30	3.2	5.051	2.19	6.002	30.1	0.155	0.027	0.124	0.36	Trace.
Small dose of phosphoric acid.	KCl, 0.03g. K_2HPO_4 0.005g.	49	2.0	0	1.43	1,43	15.09	0.175	0.032	—	1.30	10.5
Excess of phosphoric acid.	KH_2PO_4 0.03g. K_2HPO_4 0.03g. Na_2HPO_4 0.2g.	30	5.4	3.87	1·72	4.64	23.15	0.162	0.028	—	0.48	0
Small dose of potash.	Na_2HPO_4 0.06g. KCl 0.005g.	49	3.5	5.37	3.77	7.21	39.8	0.805	0.050	0.27	0.70	1.62

Experimental.

The fungus used for the purpose was Rhizopus G.34. Yamazaki, and the temperature of the culture was 28–31°C.

The culture media were prepared with several different doses of potassium or phosphoric acid, as indicated in the above table, and the other constituents(1) common to all were;- glucose 20g., $(NH_4)_2SO_4$ 1g., $MgSO_4$ and $CaCl_2$ each 0.02g., Fe_2Cl_6 and NaCl each trace, $CaCO_3$ 6g., distilled water 200 c.c.

Fumaric acid. For the estimation of the acid, a part of it was separated as calcium salt, which appeared as crystalls already in the culture medium.

---

(1) The chemicals used for this preparation are all of the Maerk's brand.

This calcium salt was recrystallized from water and dried at 120°C. The other part dissolved in the medium was extracted as in a common way with the acidification by sulphuric acids after being concentrated the medium under the reduced pressure. The other organic acids than fumaric acid, in the ether extract, was dissolved by treating the mixture with a small quantity of water, remaining behind fumaric acid almost in a pure state.

Lactic acid. The filtrate from fumaric acid was neutralized accurately with n/10 baryta water and after evaporating under the reduced pressure to a thick mass, an excess of alcohol of 80 % (v), was poured in to get into the solution the lactate of barium. The precipitate formed after a period of 12 hours was separated by the filtration, and the filtrate was evaporated under the reduced pressure to get lactate in a crystalline from. The crystallized lactate was washed several times and was determined as aldehyde after Ripperis method.

Succinic acid. The said barium salts, under lactic acid separation, which have gone to precipitation by 80 % alcohol, comprise succinate, malate and a trace of fumarate. To isolate succinic acid⁽²⁾ from the contamination, malic acid was decomposed by potassium permanganate of 5% solution, adding drop by drop to a warm solution of the salts, until the red colouration of the reagent stand persistent. Further, to this solution 5 c.c. of the reagent viz. an excess of it was added and after a period of 15 minutes, in a cooled state the excess of the reagent was removed by SO_2, and then acidifying by sulphuric acid the free acid was extracted by ether as done in a common way.

Succinic acid, thus obtained gave melting point of 183°C (uncorrect).

Malic acid. To identify malic acid, barium salts which were insoluble in alcohol of 80 v. % was acidified with sulphuric acid and extracted with ether. From the ether extract the acid was identified after Dakin's⁽³⁾ method. Cinchonin–*l*–malate thus obtained melted at 199°C.

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(2) Refar to R. Kunz's method (1903.)

(3) Dakin : Journal of Biol, Chem. Vol. LIX, No. 1., p. 7. 1924.

ON THE PHYSIOLOGY OF RHIZOPUS SPECIES.
PART IV.
THE RELATION BETWEEN THE CARBON SOURCE AND THE PRODUCTION OF ALCOHOL AND ACIDS, ESPECIALLY THE FORMATION OF SUCCINIC ACID FROM GLYCERINE.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received Feb. 15th., 1927.)

The formation of both alcohol and fumaric acid by Rhizopus species from carbohydrates, such as glucose, saccharose, starch as well as from tartaric(1) and gluconic acids(2) was already confirmed by the systematic way of researches. It was also reported by us the formation of alcohol from fumaric acid by this fungus.(3)

As to the formation of lactic acid(4) by this species not only from carbohydrates such as glucose, sucrose and starch but also from fumaric acid was mentioned in the former report.

As for the formation of succinic acid, by this fungus, in regard to its carbon source, is quite analogous to that of the case of fumaric acid, with only one exception of acetic acid, which contribute as carbon source just for the former acid.

A further research was made on the formation of alcohol and organic acids from other carbon sources than we have already concerned, by this fungus, and the authors were able to find out some unusual or singular instances in the formation of alcohol. The results were that, all carbohydrates tried were suitable for the formation of the alcohol; although a trace was to be found in the cases of such sources as xylose, fructose, lactose or inulin. This is rather a singular phenomenon, since galactose gives a moderate quantity of alcohol and sucrose a rather large quantity of it. The former may nothing more than the absence of an enzyme lactase in fungus, but the latter phenomenon finds its explanation just by the assumption that fructose, at status nascendi, behaves quite different from common fructose; because sucrose gives almost an equal quantity of alcohol as maltose does, or gives rather larger quantity of it than glucose, in as much as the doses of these carbon sources are equal.

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(1) The Journ. of Agric. Chem. Soc. of Japan. Vol. II, No. 5, p. 63 and 62.
(2) This Same Journal.
(3) Do. Vol. II, No. 5, p. 62.
(4) This Journal.

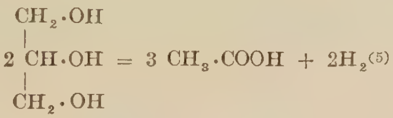
In the medium, containing glycerine as only carbon source, there was no formation of alcohol; although more or less growth of fungus was to be observable.

Fumari acid. This acid was able to be formed from all carbohydrates used, beside lactose, but from glycerine none of it was formed.

Succinic acid. This acid was found in all culture media, containing carbohydrates we have concerned, except those contributing as carbon source such one like inulin, galactose or xylose.

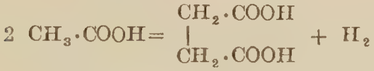
The formation of succinic acid from glycerine by this fungus is a rather noteworthy fact and its mechanism may be representable by the following way :–

1)



$$2\begin{array}{l} CH_2\cdot OH \\ | \\ CH\cdot OH \\ | \\ CH_2\cdot OH \end{array} = 3\,CH_3\cdot COOH + 2H_2{}^{(5)}$$

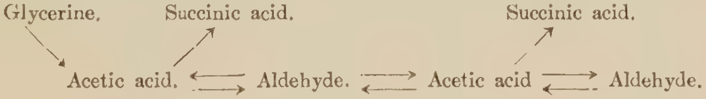
2)



$$2\,CH_3\cdot COOH = \begin{array}{l} CH_2\cdot COOH \\ | \\ CH_2\cdot COOH \end{array} + H_2$$

If we remind formation of aldehyde in such a case the third equation must comes forth :–

3) $2\,CH_3\cdot COOH = 2\,CH_3\cdot COH + O_2$ Then comes forward :–



Malic acid. This acid was formed from glucose, fructose, mannose, sucrose, maltose and starch but not from xylose, galactose, lactose, dextrine, inulin or glycerine. Upon the whole, the nature of the formation is almost equal as we have experienced in the case of succinic acid, except lactose, dextrine and glycerine.

Lactic acid. The formation of this acid from various carbon sources by this fungus is quite analogous to that of fumaric acid, except this acid may take lactose as its carbon source, when such a source could not play role for the formation of fumaric acid.

The results are tabulated below :–

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(5) Refer to Takahashi and Asai's article "on the formation of acid by Rhiz. sp. part. IV." This Journal.

Carbon Sources.	Starch.	Inulin.	Dextrin.	Sucrose.	Lactose.	Maltose.	Glucose.	Fructose.	Galactose.	Maunose.	Xylose.	Glycerine.
Weight of fungus. (g.)	0.187	0.121	0.264	0.182	0.013	0.179	0.138	0.119	0.145	0.151	0.179	0.080
Alcohol (g.) in 100c.c.(6)	0.12	Jod. r. +	0.08	0.20	trace.	0.22	0.15	Trace.	0.10	0.18	Trace.	/
Volatile acid c.c. of $^{n}/_{10}$ NaOH. for 20c.c.		0.5	0.4	/	0.1	0.2	/	0.4	0.5	0.6	0.6	0.10
Fumaric acid. m.p. and etc.	Form. report.	284°C	284°C	Form. report.	/	(7) Trace.	Form. report.	285°C	(8) 284°C	(9) 285°C	(10) 285°C	/
Succinic acid. m.p. etc.	182–183°C	/	Trace.	183°C	(11) Trace. 183°C	183–185°C (12)	Form. report.	Trace.	/	183°C	/	(13) Trace 183°C
Malic acid.	+	–	–	+	–	+?	+	+	–	+	–	–
Lactic acid.	+	+	+	+	+	+	+	+	+	+	+	– ?

Experimental.

The fungus used was Rhizopus G. 34. Yamazaki, and the culture media were prepared in such a way that each 50c.c. of the solution of mineral matters(13), containing 0.125g. of glycocoll and 1g. of $CaCO_3$ was taken in each time and to this solution any carbon source desired was added in a dose of 5%.

After a period of the culture of 16 days at 28–30°C, acids were determined or detected.

Fumaric acid. To determine this acid, it was derived into its calcium salt and calcium was precipitated as calcium oxalate, which was titrated by $^{n}/_{10}$ potassium-permanganate solution after acidified by hot hydrochloric acid of 5%. Where, 1c.c. of the reagent of permanganate used corresponds to 0.004g. of calcium or 0.0116g. of fumaric acid.

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(6) 1c.c. of $^{n}/_{10}$ potassium permanganate corresponds to 0.0116g. of fumaric acid.

(7) This substance gave positive reaction of β-naphtol + H_2SO_4 and resorcine + H_2SO_4. (Author's reactions.)

(8) It amounted to 0.15g.

(9) Its amounts were 1.20g.

(10) It amounted to 0.10g.

(11) 4.9g. of lactose gave 0.61g. of succinic acid.

(12) From 8.3g. of glycerine, there was formed 0.105g. of succinic acid.

(13) Mineral matters employed throughout our researches.

Separation of succinic and fumaric acids. In the residue of alcohol determination, the precipitate which formed was collected when hot and after washing with boiling water, was treated by ether to extract succinic acid, provided the acidification of the fluid by H_2SO_4. The other part of succinic acid was isolated from the filtrate from above precipitate, by common means of ether extraction ; where fumaric acid comes together with succinic acid. The easy soluble property, in water, of the latter acid affords us very simple way of separation from fumaric acid in the mixture.

Detection of lactic and malic acids. About the ether extract above stated, malic acid was detected by Denige's and author's reaction,(14) and lactic acid by well known Hopkin's reaction or by the formation of iodoform.

(Jan. 30. 1927.)

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(14) β-Naphtol and H_2SO_4. J. of Agric. Chem. Soc. Jap. Vol. I, No. 14.

MICROBIOLOGLCAL INVESTIGATION ON THE SOIL AND SEA-WATER FROM OGASAWARA ISLANDS. I.

By Hisamoto NAKAGAWA and Satio ARAKAWA.

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(Received Oct. 20th., 1926.)

The Ogasawara Islands are interesting from historical as well as biological point of view. The Islands came in the Japanese possession only a half century ago; and inhabited by the descendants of different nationalities. Geologically the Islands are volcanic, and in fact, one of the Islands named Iwojima (Sulfur Island) has sulfur spring and deposit all over the island, and many craters are found. The earth surface is so hot that one can hardly walk barefooted. Geographically the Islands are located far south of the Tokyo Bay in the Pacific, almost in a tropical zone having the mean temperature of 22.4°C throughout a year. Consequently it was anticipated that there may be some interesting findings among the flora of microorganisms, and one of the authors visited the Islands at the begining of this year and brought back the samples about which this report is concerned.

The samples of soil were taken from seven localities and three samples of sea-water, as indicated on the map, and the quantitative and morphological examination of microorganisms together with the determination of hydrogen ion concentration were made, and the following results were obtained:

1. PH values of the soil samples lie between 5.26 to 7.68. It is noteworthy that the soil E which was taken near the crater showed the highest hydrogen ion concentration.

2. PH values of the sea-water lie between 7.32 to 7.64 which are about the same with those values found elsewhere.

3. The quantitative determination of microorganisms indicated the presence of 9,000–701,600 on the nutrient agar plates; 524,600–7,415,800 on soil extract agar. Besides these culture medium, Ashby agar medium was used and found some organisms which made good growth.

4. All the organisms isolated were Gram positive. It was observed that many of those came from the soil were rod shaped while mostly coccic, from the sea-water.

The further report on this investigation will appear in future.

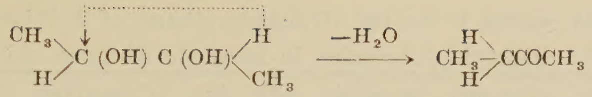
ON THE PINACOLINE TRANSFORMATION OF 2. 3–BUTYLENEGLYCOL AND ITS IDENTIFICATION.

By Tomotsune Taira.

(Received March. 2nd., 1927.)

That α-glycol, on boiling with dilute sulphuric acid, yields ketone by splitting one molecule of water and an alkyl group migrating to the neighboring carbon atom, is generally known under the name of pinacoline transformation of the same.

This reaction was first obserbed by Butlerow (A. 170 162 (1872)) on pinacone, followed by many authors on compound of more or less complex build, but hitherto no mention is made in the literature about the transformation of 2. 3-butyleneglycol. The author (This Journ. **25**, 734, 1926), in the course of his investigations on the occurence of 2. 3-butyleneglycol in Japanese fermentation products, happened to observe that methyl-ethyl-ketone always accompanied 2. 3-butyleneglycol on distilling such products with dilute sulfuric acid. This ketone must have been produced according to the following scheme and might be regarded as a simple example of the pinacoline transformation.



2. 3-butyleneglycol methyl-ethyl-ketone

Since methyl-ethy-ketone can readily be estimated as its *p*-nitrophenylhydrazone, 2. 3-butyleneglycol can also be identified by preparing the same.

EXPERIMENTAL.

Preparation of 2. 3-butyleneglycol.

2. 3-butyleneglycol was extracted from soya-sauce and saké in the following way.

A quantity of saké (or soya-sauce) was evaporated to a syrupy consistency under diminished pressures and it was then extracted with ether after being made slightly alkaline with caustic soda solution. The ethereal extract was dried, ether driven off by distillation and the residue thus obtained was subjected to the fractional distillation in vacuo, when a glycerine like substance having the characteristic odour of those fermentation products came over at 70–80°C under a pressure of 5–6m.m. mercury. After being rectified by repeated vacuum distillations, the substance distilled almost constantly at 170–180°C. under normal pressure, with a density 1.004 and n_D^{20} 1.4300.

0.1104 g. of substance gave 0.2151 g. CO_2 and 0.1048 g. H_2O
0.2754 g. of substance gave 0.5334 g. CO_2 and 0.2496 g. H_2O

	C 53.13 %	H 10.54 %
	C 52.83 %	H 10.07 %
$C_4H_{10}O_2$ requires	C 53.33 %	H 11.11 %

The molecular weight determined by the freezing point method using glacial acetic acid gave the following results.

substance (g.)	solvent (g.)	depression of freezing point	molecular weight
0.2240	21.2900	0.498	
		0.526	
		0.520	
		mean 0.515	79

The same by the elevation of boiling point using methyl alcohol was,

substance (g.)	solvent (g.)	ebullition of boiling point	molecular weight
0.2600	15.277	0.180	80
	Calculated from	$C_4H_{10}O_2$	90

Preparation of diphenylurethane.

The mixture of 1c.c. of the substance with 2c.c. of phenylisocyanate and 5c.c. of benzene was warmed on the flame, and on standing, the formation of needle crystals was obserbed, which after due purification melted at 200° and was proved to be the diphenylurethane of 2, 3-butyleneglycol.

Oxidation to diacetyl by bromine in presence of ferric chloride.

0.5g. of the substance together with 2c.c. of bromine, 0.3g. of ferric chloride and 5g. of sodium acetate were heated on the water bath for 20 minutes, the excess of bromine was removed with sodium bisulfite sotution and then the residue was distilled on the oil bath. The distillate represented a floating oily substance having a very pungent odour. One drop of the distillate taken in a test tube and 1c.c, of 20 % hydroxylamine hydrochloride solution and 2c.c. of 20 % sodium acetate solution were added. After little boiling, it gave a apecial carmine coloured needle like crystal of nickel-dimethylglyoxime on adding a few drops of 10% nickel chloride solution(Kluyver and his coworker ; (Biochem. Zts. 161 361 (1926)). To the residue of the above test water solution of hydroxylamine hydrochloride was added and neutralized with sodium carbonate. On standing some time white crystal were obtained, which was filtered and washed with water and dried in a desiccator. The crystal showed a melting point of 234°–235°C and found to be the diacetyldioxime related by Wolff (A 288 27). Dehydration with 25% sulphuric acid.

0.5g. of the substance was taken in a distillation flask, added 30c.c. of 25% sulphuric acid and distilled on the oil bath until the thermometer of the bath indicates 180°C. The distillate was cooled by the freezing mixture and 20c.c. of aldehyde like odoured liquid was obtained. On mixing the distillate with 1/2 volume of acetic acid and 30 % acetic acid solution of *p*-nitrophenylhydrazine, yellow needle crystals soon appeared, which was filtered and washed with 39% acetic acid and recrystallized from 40% ethyl alcohol. The melting point of it was 130°C.

Analysis 0.0825 g. of the substance gave 15.4c.c. N (31°C 755 m.m.)

Fonnd	20.29%
Calculated from $C_{10}H_{13}N_3O_2$	20.18%

The above results closely ressmble to isobutylaldehyde-*p*-nitrophenylhydrazone $(CH_3)_2\,CHCH : NNHC_6H_4NO_2$ (m. p. 130°–132°C) and also to its isomeric methyl-ethyl-ketone-*p*-nitrophenylhydrazone $CH_3CH_2C: NNH\ C_6H_4NO_2$ CH_3 (m. p. 128–129°C). When the substance mixed with the former, they showed a melting point of 103°C, and with the latter, they showed no tendency of depressing the melting point.

SUMMARY.

1. Observed the pinacoline transformation of 2. 3-butyleneglycol.
2. 2. 3-butyleneglycol can be identified by the dehydration with 25% sulphuric acid, namely by the formation of methyl-ethyl-ketone-*p*-nitrophenylhydrazone.

(Government Research Institute. Formosa.)

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